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An Evaluation of Cystatin C Levels in the Serum and Urine as Early Diagnostic for Iraqi Patients with Type 2 Diabetes

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Abstract

The current study the effect of urine and serum cystatin in diabetic nephropathy patients in Najaf. This investigation was carried out at the Al-Sadr Teaching Hospital in Najaf's Diabetes and Endocrine Center for a period from May 2022 to October 2022. the study included 42 males with diabetic type 2 ranging in age from 30 to 62. they were divided into 3 groups intended UACR and were divided, into two groups depending on Egfr and evaluated serum and urine cystatin C, HbA1C, creatinine, urea, UACR, Egfr, lipid profile, CRP. The result of the current study according to albuminuria showed no significance in age, duration of diabetes, BMI, cholesterol, HDL, VLDL, while this study registered significance ($p<0.05$) in HbA1C, UACR, Serum CR, eGFR, urea, TG, CRP, serum cystatin and urine cystatin. So, when comparing eGFR stages were found higher significance($p<0.001$) in UACR, eGFR, and ($p<0.05$) serum CR, Urea, TG, CRP, serum cystatin, and urine cystatin. ROC for cystatin C was 0.703 and ROC for urine cystatin was 0.802. The negative relationship between eGFR and urine cystatin C levels, serum cystatin C levels and (serum creatinine) {$R = -0.438$, $p = 0.003$}, {$R = -0.416$, $p = 0.010$ }, { $R = -0.557$, $p = 0.009$}. in conclusion Urine and serum cystatin maybe use a marker besides creatinine to diagnose early kidney dysfunction.

Keywords
Diabetic Nephropathy, Urine Cystatin C, Serum Cystatin C.

One of the most common diabetic microvascular problems is known, as diabetic nephropathy (DN), which is distinguished by a slow increase in the rate of albumin excretion in the urine along with arise in blood pressure, and a sharp fall in the glomerular filtration, rate ultimately Leading to the stage end of ESKD renal failure (1).

A non-glycosylated, basic protein with a small molecular weight is called cystatin C. (13 kDa).(2) It is a member of the type 2 cystatin superfamily is cystatin C (Cys-C) (3). Post gamma or gamma-trace is a single-chain, that makes up to 4% of the protein in cerebrospinal fluid (CSF) and whose serum concentration is closely correlated with the GFR(4) . It has a string of 120 amino acids, is created by nuclear cells at a constant pace, and is found in rise amounts in biological fluids (5) (6). On chromosome 20, there is a gene that produces cystatin C. Cystatin C is a substance which is found in the systemic circulation at high amounts and is regarded as an important cysteine protease extracellular inhibitor with potent antiviral action(7). It is stable in nearly all nucleated cells. The glomerular filtration that is most correlated with cystatin C concentration is regarded as unaffected by other factors (nutrition, infections, liver health, cancer, myopathies, and body fat

percentage). The currently used glomerular filtration indicator of renal function is serum creatinine. however, it can be influenced by factors like, age, race, gender, health status, and body mass (8). These elements are considered in into account in equations for estimating the glomerular filtration rate (GFR), such as" the Modification of Diet in Renal Disease. (MDRD) formulas" to calculate physiological aspects that impact creatinine. Cys-C has shown promise as a diagnostic marker in earlier studies. (9) showing a close relationship to GFR and strong sensitivity independent of minor, moderately, or severe renal impairment. Aincreasing body of research indicates that Cystatin C can be used to identify, renal disease earlier than serum creatinine which may aid in preventative, efforts in elderly individuals as well as those with diabetes hypertension or cardiovascular, disease . It also suggests a link between cystatin C and amyloid-related diseases like Alzheimer's disease (10).

Among those with type 2 Diabetes mellitus , the amount of urine cystatin C has been established as an indicator of early kidney impairment (11). In a more recent investigation, it was discovered that urine cystatin C levels were associated with urine ACR favorably both pre - diabetes and diabetes and were considerably greater in patients with microalbuminuria compared to normal luminaria. Elevated urine cystatin C was one of the earliest indicators of diabetic and pre −diabetic, nephropathy indicating that the Development of nephropathy in pre − diabetes perhaps significantly influenced by cystatin C (12) . The study's results indicate that, urine Cystatin C levels probably agood diagnostic for microalbuminuria detection regardless of other tubular indicators and irrespective of the degree of microalbuminuria in early DN (13). and independent of other tubular indicators and the degree of tubular dysfunction, may be a valuable diagnostic for the identification of microalbuminuria (13). The study's objective was to evaluate how well Cystatin-C levels in the blood and urine may be used to detect nephropathy in Diabetes Mellitus II early on.

Material and Methods

The 42 adult male individuals who visited with type 2 Diabetes at the diabetes and Endocrine center at Al - sadr teaching hospital in najaf ranged in age of 30 to 62 . Those are The research was conducted from May 2022 to October 2022. To simplify the Collection of samples, official, approvals were sought from the Najaf Health Department, and patient consent was also secured. Based on the urine albumin to creatinine ratio, the patients were separated into three groups(14); normoalbuminuria (> 30) (N=26), a microalbuminuria stage from (30-300)(N=8), and the macroalbuminuria of (300-3000)(N=8). In addition, two stages were created for the patients based on the glomerular filtration rate calculated using the MDRD equation; "(GFR > 60 mg/min *1.73)" (N =24) and "GFR<60 mg/min *1.73)" (N= 18). Five milliliters of venous blood were collected, two milliliters of that volume were put in tubes with EDTA to calculate the HbA1C. The remainder (3 ml) was then centrifuged at 5000 revolutions per minute for 5 minutes with the residual volume at room temperature for 30 minutes to get the serum. The urine and serum Cystatin C concentrations were assessed using the Elisa Technique from the Elabscience firm, and the UACR and HbA1C levels were calculated using the Cobas e411 and COBAS C111 Analyzers, respectively. In addition, a spectrophotometer from the BioSystem firm analyzed the lipid profile, urea, and creatinine levels (10). While in renal disease, GFR was calculated using dietary changes. "186*(serum creatinine (mg/dl) $^{-1.154}$*Age $^{-0.203}$" is the MDRD abbreviation(15). "The Chronic Kidney Disease Epidemiology (CKD EPI) equation: (16). was used to get the eGFR level. Furthermore, SPSS version 23 was used to conduct the statistical analysis. The study's samples were evaluated using mean and standard deviation for values that were normally distributed. For values that were not normally distributed, ANOVA was used to analyze group differences before the test Kruskal-Wallis. Utilizing the Spearman correlation coefficient, the connection between urine & serum cystatin C, serum CR

and eGFR. To establish cutoff values for diagnosing kidney dysfunction in diabetes patients, the under area the cystatin C curve in the serum and urine was calculated using the Receiver Operating Characteristic (ROC) Analysis.

Result & Discussion

The urinary albumin / creatinine ratio (ACR) was used to classify patients into normoalbuminuric, microalbuminuric, and macroalbuminuric groups based on their excretion of urinary albumin (Table 1). displays the subjects' initial characteristics. Age, duration and BMI did not differ in a significant statistical way ($p > 0.05$). In the present research, the HbA1C levels in the three groups varied significantly (p=0.028). High significance was seen in ACR levels ($p = 0.001$). However, eGFR in the macroalbuminuric group (52± 11) was considerably inferior to in the microalbuminuric (58.7± 9.1) and normoalbuminuric (79 ±12.73) groups (p 0.009) estimated in MDRD and (p= 0.006) in EPI, substantial differences in serum CR (p= 0.032), urea(p= 0.003),cholesterol (p=0.008),TG(p=0.002) and CRP levels($p = 0.006$) were seen in the three groups, urine and serum cystatin was showed significant in macroalbuminuric (p= 0.047),(p= 0.040) (Table 1).

According to Egfr by the MDRD equation (Table 2) showed no significant in age, duration, BMI, HbA1C, cholesterol, LDL, VLDL, while higher significant in UACR(p=0.005),Egfr calculated by the EPI (p=0.001), serum CR(P=0.026),urea (p=0.018), HDL(p=0.020),TG(p=0.024), CRP (p=0.035),serum cystatin (p=0.032),urine cystatin(p=0.043).

levels of cystatin C in urine and serum were utilized to construct the diagnostic profile for eGFR 60 mL/min/1.73 m^2 between type 2 diabetic subjects utilizing the region under "Receiver Operator Characteristic" ROC tests. The levels of cystatin C in the urine confirmed the diagnosis., showing an of AUC 0.802 (95 % CI 0.590-0.998) with a worth of cutoff of 399ng/ml with a sensitivity of 96 % and a specificity of 75 %. Cystatin C levels in the serum displayed an AUC of 0.703 (95 % CI 0.512-895) with a cutoff of 325 ng/ml with a sensitivity of 84 percent and a specificity of 67 percent. (Fig.1). According to the current results, urine cystatin C levels and eGFR have a significantly associated, negatively linear relationship ($r = - 0.438$, $p = 0.003$, fig. 2) Additionally, the study revealed a negative correlation between eGFR and serum cystatin C levels ($r = - 0.416$ $p = 0.010$, fig.3).and this study revealed a negative correlation between eGFR and serum creatinine ($r = - 0.557$, $p = 0.009$, fig 4).

Table1: lists the characteristics and certainbiochemical parameters of diabetic nephropathy patients at various stages.

Parameters	Normo-albuminuria	Micro-albuminuria	Macro-albuminuria	P value
Age(yr)	50.84±9.37	50.75±8.46	51±3.36	0.99
Duration (yr)	7.38 ±5.60	10±9.23	9±2.7	0.718
BMI (kg/m^2)	27.4±4.15	28.05±2.55	30.10±3.05	0.47
HbA1C (%)	8.02±1.05	9.50±1.13	10.5±1.96	0.028
UACR (%)	12.0±4.59	75.25± 12.89	627±353	0.001
“eGFR calculated by the CKD-EPI equation ($ml/min.1.73m^2$)”	79±12.73	66±12.17	53±11	0.006
“eGFR is calculated by the MDRD equation $ml/min.1.73m^2$)”	70.7±10	58.7±9.1	52±11	0.009
Serum Cr (mg/dl)	1.10 ± 15	1.35±0.10	1.37±0.4	0.032
Urea (mg/dl)	25.6±5.63	33.25±4.34	36.50±3.78	0.003
Cholesterol (mg/dl)	171.15±55.24	248±29.86	257±23.13	0.008
LDL (mg/dl)	86.9±36	106.25±23.37	128.5±21.9	0.227
HDL (mg/dl)	45.6±22.7	29.25±24.6	21.5±2.1	0.240
TG (mg/dl)	157±46.92	348±122.44	355±109	0.002
VLDL (mg/dl)	38.84±19.29	70±51.2	42±7.07	0.459
CRP (mg/dl)	3.96±3.27	5.21±2.7	19.4±12.8	0.006
Serum Cystatin (ng/ml)	276.8±140.27	380±46.6	421.25±47.35	0.047
Urine Cystatin (ng/ml)	348.2± 98.17	439.25±25.5	454.75±3.09	0.040
For continuous variables, data are reported as mean± SD. P<0.005 in an ANOVA test is regarded as significant, and P<0.001 as very significant.				

Table2.The MDRD eGFR stage effect of some parameters in type2diabetes patients

Parameters	eGFR>60	eGFR<60	P value
Age (yr)	49±8.7	54±5.3	0.270
Duration (yr)	8.4±6.6	7.6±3.5	0.938
BMI (kg/m^2)	27±3.9	30±2.3	0.139
HbA1C (%)	9.25±2.5	10±1.7	0.671
UACR (%)	25.3±28.6	430±409	0.005
"eGFR calculated by the CKD-EPI equation (ml/min.1.73m^2)"	82.6±9.8	72.5±8.09	0.001
"EGFR calculated by the MDRD equation (ml/min.1.73m^2)"	57.7±10.6	54.5±9.4	0.001
serum CR (mg/dl)	1.1±0.14	1.38±0.3	0.026
Urea (mg/dl)	27±6.02	34±5.92	0.018
Total cholesterol (mg/dl)	189±59.7	230±60.5	0.200
LDL (mg/dl)	89±34.6	119±23.25	0.120
HDL (mg/dl)	45±22.04	16±6.13	0.020
VLDL (mg/dl)	48±32	37±7.13	0.689
TG (mg/dl)	200±111	307±114	0.024
CRP (mg/dl)	4.4±3.2	14±13	0.035
Serum Cystatin (ng/ml)	312±119	414±13	0.032
Urine Cystatin (ng/ml)	361±97	448±17	0.043
For continuous variables, data are reported as mean± SD. P<0.005 in an ANOVA test is regarded as significant, and P<0.001 as very significant.			

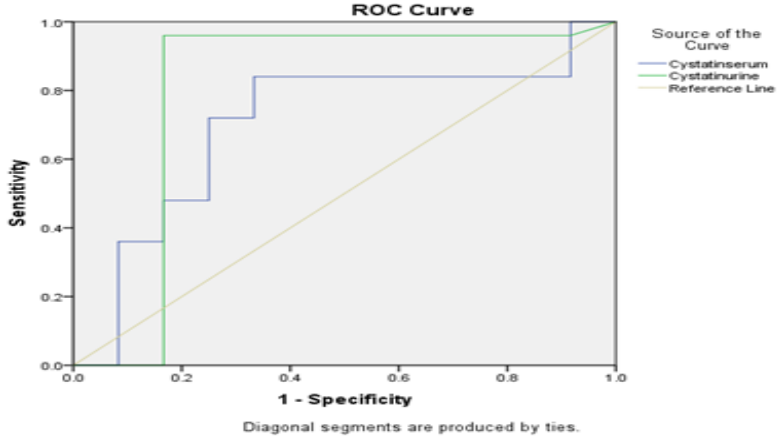

Fig1. Cystatin C RUC curves in urine and serum about Egfr of 60 ml/min/1.73 m^2 were studied using the MDRD equation. The area for serum cystatin C was 0.703 (95% CI 0.512-895) and for urine cystatin, C was 0.802 (95 % CI 0.590-0.998). The cutoff values for urine cystatin C were 399ng/ml with a sensitivity of 96% and specificity of 75%, whereas the cutoff values for serum cystatin C were 325 ng/ml with a sensitivity of 84 and 67%, respectively.

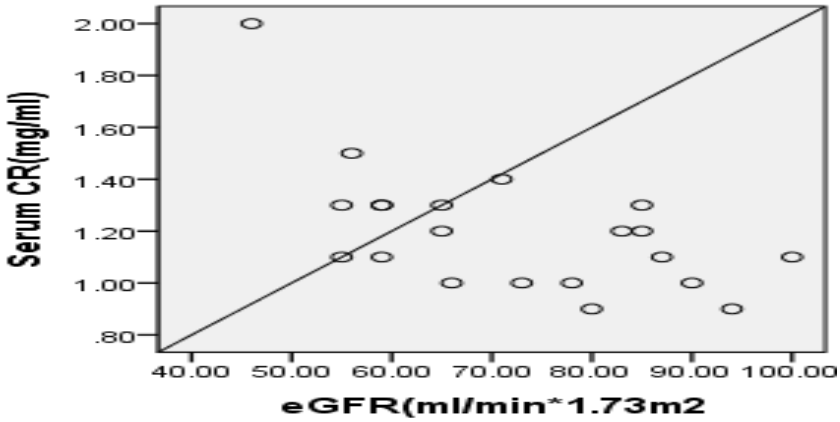

Fig1.The relationship between serum Creatinine and eGFR

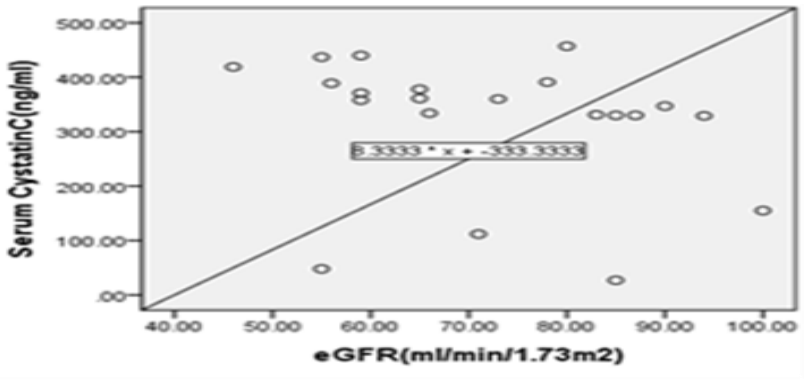

Fig2. The relationship between serum CystatinC and eGFR

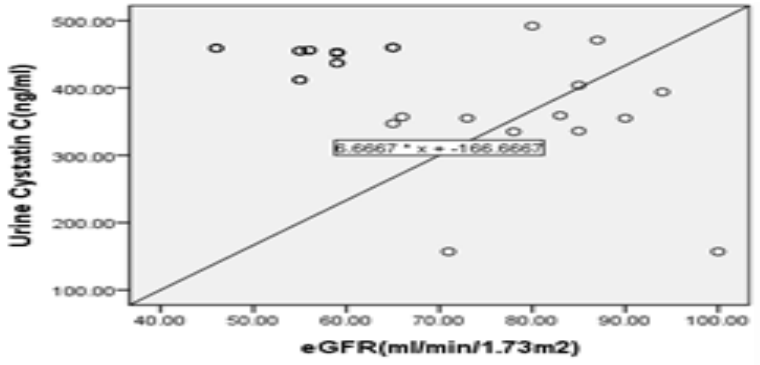

Fig3.Relationship between urine Cystatin C and eGFR

We assessed the amount of cystatin C in type 2 diabetic individuals by putting them into 3 groups based on: the varying degrees of renal impairment.
(normoalbuminuria,microalbuminuria, and macroalbuminuria). In comparison to individuals with normoalbuminuria and microalbuminuria, patients with macroalbuminuria had considerably higher Cystatin C concentrations in the serum and urine. We also discovered that serum and urine cystatin C levels were greatly greater in people with GFRs below 60 ml/min/1.73 m^2 than in people with GFRs above 60 ml/min/1.73 m^2. This consistent with study (17). This increase was believed to be the result of tubular development before glomerular appearance. This implies that subclinical tubular impairment and serum and urine cystatin C levels are connected (17).
Cystatin C is free filtration through the glomerulus, not released through tubule, and is not removed through any further renal pathways. Instead, it is just about entirely absorbed and digested by proximal tubular cells. Cystatin C is a superb GFR biomarker since the GFR nearly entirely controls the plasma levels of this protein. Compared to standard clinical measurements of renal function, cystatin C offers advantages. It is more trustworthy than the 24-hour creatinine clearance and more accurate than the Cockcroft-Gault estimate of creatinine clearance and plasma creatinine. A growing amount of research suggest that cystatin C possibly more efficient than serum creatinine in the early diagnosis of renal disease., which may aid preventative efforts in the elderly and people with certain medical conditions (18) .
According to the study's findings, urine cystatin C levels can serve as a reliable indicator of microalbuminuria in the early stages of DN and can diagnose the condition independently of other tubular markers and the degree of tubular dysfunction (13). In a different study of people with type 2 diabetes, A better indicator of early nephropathy was discovered to be increased urine cystatin C excretion. This was because increased urine cystatin C excretion was linked to a drop in GFR, especially in patients with DN who had an eGFR of less than 60 ml/min/1.73 m^2 in the early stages. greater urine excretion of cystatin C was a stronger indicator of early nephropathy, according to. Urinary

cystatin C perhaps used as a biomarker for determining how type 2 diabetics' nephropathy will develop because tubular damage (19).
Additional recent research investigated the link between albuminuria and cystatin C levels in addition the clinical value of serum and urine cystatin C levels in forecasting kidney dysfunction in normoalbuminuric type 2 diabetes patients. Patients with type 2 diabetes whose albuminuria excretion is normal, serum and urine cystatin C levels may be a valuable marker for predicting early nephropathy. This was suggested by the finding that Patients with normoalbuminuria had an independent relationship between eGFR of 60 ml/min/1.73 m2 and serum and urine cystatin C levels. The usefulness of cystatin C as a biomarker in serum or urine to identify early nephropathy in people with normoalbuminuria [early nephropathy] and in those with type 2 diabetes who have normal-, micro-, or macroalbuminuria to predict the evolution of nephropathy is demonstrated by the serum and urine cystatin C levels increasing as the degree of albuminuria increased in patients with macroalbuminuria. Specifically, among people with normoalbuminuria, Detecting cystatin C in urine and serum, according to the study's authors, is a practical, non-invasive, and effective technique to track renal involvement as diabetes progresses (17). Some studies showed that blood creatinine fails to adequately diagnose a renal failure in older people, those with diabetes, injuries to the spine, and people who have liver cirrhosis and that cystatin C is superior indicator of renal function(3). Also in Table(1)showed a significant increase in HbA1C, UACR, Egfr, serumcr, urea, CRP agreed with the study(20).

Conclusions

The summery of the present results, cystatin C measurement in urine and serum is an indicator of early identification of diabetic nephropathy There perhaps certain restrictions on our investigation. Due to the study's limited sample size and the fact that patients were not requested to stop taking any medications, including
antihypertensive drugs, participants were not asked to do so. As a result, albuminuria in these patients might be underdiagnosed. But there is some merit to this study. Urine & serum Cystatin C levels were assessed simultaneously. Additionally, this study made it abundantly evident that albuminuria and serum cystatin C levels were both increased in diabetes individuals. The usefulness of serum cystatin C for the measurement of GFR requires more research in various clinical contexts with a larger sample size of patients and healthy people, as well as in-depth examinations of any illnesses or drugs that could affect cystatin C levels. This study's findings suggest that cystatin C detection in urine and serum is a trustworthy, non-invasive method for figuring out involvement of the kidneys in development of diabetes, particularly in normoalbuminuric patients. Cystatin C probably a helpful an indicator of the early diagnosis of diabetic nephropathy, however further research To confirm this, a bigger sample size and a prospective design are needed

References

Kittell F. Diabetes Management. Thomas LK, Othersen JB Nutr Ther Chronic Kidney Dis CRC Press pp. 2012;198.

Bengtsson E, To F, Haǩansson K, Grubb A, Braǹǐn L, Nilsson J, et al. Lack of the cysteine protease inhibitor cystatin C promotes atherosclerosis in apolipoprotein E−deficient mice. Arterioscler Thromb Vasc Biol.2005;25(10):2151−6.

Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. Nephrol Dial Transplant.2006;21(7):1855−62.

Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007;130(3):456−69.

Gashenko EA, Lebedeva VA, Brak I V, Tsykalenko EA, Vinokurova G V, Korolenko TA. Evaluation of serum procathepsin B, cystatin B and cystatin C as possible biomarkers of ovarian cancer. Int J Circumpolar Health. 2013;72(1):21215.

Benson K, Cramer S, Galla H-J. Impedance-based cell monitoring: barrier properties and beyond. Fluids Barriers CNS. 2013;10(1):1–11.

Saitoh E, Sabatini LM, Eddy RL, Shows TB, Azen EA, Isemura S, et al. The human cystatin C gene (CST3) is a member of the cystatin gene family which is localized on chromosome 20. Biochem Biophys Res Commun. 1989;162(3):1324–31.

Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int. 1985;28(5):830–8.

Аљčić-Buturović B, Čavaljuga S. Cystatin C as a marker for detection of early renal failure in diabetes type 2 patients. Bosn J Basic Med Sci. 2005;5(4):68.

Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. Ann Intern Med. 2004;141(12):929–37.

Rao X, Wan M, Qiu C, Jiang C. Role of cystatin C in renal damage and the optimum cut-off point of renal damage among patients with type 2 diabetes mellitus. Exp Ther Med. 2014;8(3):887–92.

Garg V, Kumar M, Mahapatra HS, Chitkara A, Gadpayle AK, Sekhar V. Novel urinary biomarkers in pre-diabetic nephropathy. Clin Exp Nephrol. 2015;19(5):895–900.

Ibrahim MA, Ahmed YS, El-Shinnawy HA, Maseeh I, Makkeyah YM, Bichari WA. Value of urinary cystatin C in early detection of diabetic nephropathy in type 2 diabetes mellitus. Int J Adv Res Biol Sci. 2015;2(3):211–23.

Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med. 1999;130(6):461–70.

Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. National Kidney Disease Education Program Laboratory Working Group: Recommendations for improving serum creatinine measurement: A report from the laboratory working group of the national kidney disease education program. Clin Chem. 2006;52(1):5–18.

Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J kidney Dis. 2008;51(3):395–406.

Jeon YK, Kim MR, Huh JE, Mok JY, Song SH, Kim SS, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci. 2011;26(2):258–63.

Hirai M, Suzuki S, Hinokio Y, Hirai A, Chiba M, Akai H, et al. Variations in vitamin D-binding protein (group-specific component protein) are associated with fasting plasma insulin levels in Japanese with normal glucose tolerance. J Clin Endocrinol Metab. 2000;85(5):1951–3.

Kim SS, Song SH, Kim IJ, Jeon YK, Kim BH, Kwak IS, et al. Urinary cystatin C and tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care. 2013;36(3):656–61.

Najjar ZSR, Jwad SM, Alkhafaji RS. Assessment of 1.25 (OH) 2D3 Levels and Nephropathy Stages in Adult Male Patients with Type-2 Diabetes Mellitus.